PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Study protocol of a phase Ib/II clinical trial of metformin and chloroquine in patients with *IDH1*-mutated or *IDH2*-mutated solid tumors
AUTHORS	Molenaar, Remco; Coelen, Robert; Khurshed, Mohammed; Roos, Eva; Caan, Matthan; van Linde, Myra; Kouwenhoven, Mathilde; Bramer, Jos; Bovée, Judith; Mathôt, Ron; Klumpen, Heinz-Josef; van Laarhoven, Hanneke; van Noorden, Cornelis; Vandertop, W. Peter; Gelderblom, Hans; van Gulik, Thomas; Wilmink, Johanna

VERSION 1 - REVIEW

REVIEWER	Julia Tchou University of Pennsylvania Perelman School of Medicine Abramson Cancer Center
REVIEW RETURNED	13-Nov-2016

GENERAL COMMENTS	The objective of this Phase Ib/II clinical trial is to evaluate the maximal tolerated dose of metformin and chloroquin repurposed as anti-cancer drugs against three malignancies which do not respond well to conventional chemotherapy/radiation: chondrosarcoma, intrahepatic and glioma. All three malignancies harbor IDH1/2 mutations which render them susceptible to the metabolic effects of metformin and chloroquin. Once deemed eligible, enrolled patients will start with metformin in the first week. chloroquin will then be added in the second week. Metformin dose escalation in a 3+3 design will start at 500 mg BID. The next escalated dose is 1000 mg BID and the last escalated dose is 1500 mg BID. The higher metformin dose being tested is welcome since the efficacy of metformin as an anti-cancer drug is likely different from that as an anti-diabetes drug. Staging imaging will be performed in week 9. Patients will continue on combination therapy until disease progression occurs or until 2 days before planned resection. This clinical trial design allows maximal flexibility for clinicians to enroll patients ensuring successful patient accrual. The trial aims to enroll 20 patients with a focus to enroll patients with resectable intrahepatic cholangiosarcoma and chondrosarcoma. Pre- and post treatment tissue samples will provide valuable correlative endpoints. This is an important study targeting patients with IDH1/2 mutation who are most likely to be sensitive to the proposed drug combination. This proof of concept study will pave the way to future clinical trials to repurpose this and other FDA approved drug as novel anti-cancer drugs.

REVIEWER	Taxiarchis Kourelis

	Mayo Clinic, Minnesota, USA
REVIEW RETURNED	22-Dec-2016

GENERAL COMMENTS	The authors describe the design for a phase I/II clinical trial to identify the MTD for the combination of metformin and chloroquine in patients with IDH1/2-mutated chondrosarcoma, glioma, and intrahepatic cholangiocarcinoma. They plan to use a classic 3+3 dose escalation scheme. The concept is very interesting since the preclinical rationale is reasonable, the drugs are cheap and have been in clinical practice for decades and alternative treatment options have relatively poor efficacy and tolerability. Correlatives and pharmacokinetics are well designed. I have the following suggestions: 1) I would recommend the authors "stick to" either metformin or phenformin rather than switching to using phenformin if no activity is seen: a) this is a dose finding study primarily and not an efficacy study b) the sample size required might change if some patients get switched due to lack of efficacy. If the authors want to use phenformin this should be a in prespecified cohort in which patients get randomized to and they should account for it in their sample size and include in the consent. However the "cleanest" thing to do is stick to one drug. 2) Please clarify what method will be used to identify IDH mutations for each tumor type. If you want to use 2HG as a surrogate for some tissues that is ok but please pre-specify which method that will be and commit to it. If you have doubts about the use of this surrogate, use what is considered the gold standard in each case and keep secondary objective #3. If you do not have doubts remove secondary objective #3. Using this as an eligibility criterion and a secondary objective is confusing. 3) Please consider revising eligibility criteria for recurrent disease to patients who have failed more established treatments (e.g. temozolomide for gliomas). That is especially true for aggressive, high grade recurrent gliomas (WHO IV). 4) Toxicity monitoring. For metformin: please include periodic B12 assessments and educate patients formally about signs of hypoglycemia. Chloroquine: please include periodic ECGs. Also periodic (preferred) or as needed ophthalmologic evaluations.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Julia Tchou
Institution and Country: University of Pennsylvania, Perelman School of Medicine, Abramson Cancer Center, USA
Competing Interests: None declared

The objective of this Phase Ib/II clinical trial is to evaluate the maximal tolerated dose of metformin and chloroquin repurposed as anti-cancer drugs against three malignancies which do not respond well to conventional chemotherapy/radiation: chondrosarcoma, intrahepatic and glioma. All three malignancies harbor IDH1/2 mutations which render them susceptible to the metabolic effects of

metformin and chloroquin. Once deemed eligible, enrolled patients will start with metformin in the first week. chloroquin will then be added in the second week. Metformin dose escalation in a 3+3 design will start at 500 mg BID. The next escalated dose is 1000 mg BID and the last escalated dose is 1500 mg BID. The higher metformin dose being tested is welcome since the efficacy of metformin as an anti-cancer drug is likely different from that as an anti-diabetes drug. Staging imaging will be performed in week 9. Patients will continue on combination therapy until disease progression occurs or until 2 days before planned resection. This clinical trial design allows maximal flexibility for clinicians to enroll patients ensuring successful patient accrual. The trial aims to enroll 20 patients with a focus to enroll patients with resectable intrahepatic cholangiosarcoma and chondrosarcoma. Pre- and post treatment tissue samples will provide valuable correlative endpoints.

This is an important study targeting patients with IDH1/2 mutation who are most likely to be sensitive to the proposed drug combination. This proof of concept study will pave the way to future clinical trials to repurpose this and other FDA approved drug as novel anti-cancer drugs.

We thank Dr. Tchou for her review of our manuscript.

Reviewer: 2
Reviewer Name: Taxiarchis Kourelis
Institution and Country: Mayo Clinic, Minnesota, USA
Competing Interests: none declared

The authors describe the design for a phase I/II clinical trial to identify the MTD for the combination of metformin and chloroquine in patients with IDH1/2-mutated chondrosarcoma, glioma, and intrahepatic cholangiocarcinoma.
They plan to use a classic 3+3 dose escalation scheme.

The concept is very interesting since the preclinical rationale is reasonable, the drugs are cheap and have been in clinical practice for decades and alternative treatment options have relatively poor efficacy and tolerability.
Correlatives and pharmacokinetics are well designed.

I have the following suggestions:
1) I would recommend the authors "stick to" either metformin or phenformin rather than switching to using phenformin if no activity is seen: a) this is a dose finding study primarily and not an efficacy study b) the sample size required might change if some patients get switched due to lack of efficacy. If the authors want to use phenformin this should be a in prespecified cohort in which patients get randomized to and they should account for it in their sample size and include in the consent. However the "cleanest" thing to do is stick to one drug.
2) Please clarify what method will be used to identify IDH mutations for each tumor type. If you want to use 2HG as a surrogate for some tissues that is ok but please pre-specify which method that will be and commit to it. If you have doubts about the use of this surrogate, use what is considered the gold standard in each case and keep secondary objective #3. If you do not have doubts remove secondary objective #3. Using this as an eligibility criterion and a secondary objective is confusing.
3) Please consider revising eligibility criteria for recurrent disease to patients who have failed more established treatments (e.g. temozolomide for gliomas). That is especially true for aggressive, high grade recurrent gliomas (WHO IV).
4) Toxicity monitoring. For metformin: please include periodic B12 assessments and educate patients formally about signs of hypoglycemia. Chloroquine: please include periodic ECGs. Also periodic (preferred) or as needed ophthalmologic evaluations.

1) We thank Dr. Kourelis for his review of our manuscript. We agree with him that the cleanest clinical

trial design is with one drug. In the previous submission of our manuscript, we discussed that phenformin might be a future alternative for metformin in the case that metformin treatment fails (in future clinical trials). Phenformin is not a part of our present study protocol. We thank Dr. Kourelis for pointing this out and we have now clarified this in the manuscript.
2) We agree with Dr. Kourelis that D-2HG measurements cannot play a role in both the study objectives as well as the inclusion criteria. The study objectives and inclusion criteria now reflect this.
3) We agree with Dr. Kourelis that patients with recurrent disease who have failed established treatments should be able to enroll in our clinical trial. The inclusion criteria now reflect this.
4) We thank Dr. Kourelis for his helpful remarks. We will periodically assess serum vitamin B12 levels (every 8 weeks) and perform periodic ECGs (every 24 weeks). Table 3 now reflects this. We will also educate patients about signs of hypoglycemia (see manuscript page 20). Dr. Kourelis points out that chloroquine can induce retinopathy and suggests to perform periodic or as needed ophthalmologic evaluations. It is correct that large cumulative doses (>460 gram) of chloroquine can induce retinopathy (Bull's Eye maculopathy).1 Daily doses up to 250 mg per day for several years are considered to carry an acceptable risk for chloroquine-induced retinopathies.2 In the proposed clinical trial, patients will be treated with 200 mg chloroquine per day (cumulative dose per year: 73 grams). Therefore, the relatively short duration of this clinical trial carries a very low risk to induce chloroquine-related retinopathies. Long-term use of chloroquine (>5 years or >300 grams cumulative dose) is an exclusion criterion for this trial to prevent chloroquine-related retinopathies. We perform an ophthalmologic evaluation in case the estimated cumulative chloroquine dose of a patient exceeds 300 mg (see manuscript page 20).

References:
1. Michaelides M, Stover NB, Francis PJ, et al. Retinal toxicity associated with hydroxychloroquine and chloroquine: risk factors, screening, and progression despite cessation of therapy. Archives of ophthalmology 2011;129(1):30-9.
2. Marmor MF, Kellner U, Lai TY, et al. Revised recommendations on screening for chloroquine and hydroxychloroquine retinopathy. Ophthalmology 2011;118(2):415-22.

VERSION 2 – REVIEW

REVIEWER	Taxiarchis Kourelis Mayo Clinic, Rochester, MN, USA
REVIEW RETURNED	31-Jan-2017

GENERAL COMMENTS	Looks great. Thank you for considering the changes suggested.